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16. ABSTRACT

A significant number of chemicals have been shown to be carcinogenic in mouse liver while lacking carcinogenic activity in other organs or tissues of mice or rats. This review focus on the reasons for the unique susceptibility of the mouse liver to these carcinogens, and the extent the carcinogenic activity of a chemical in mouse liver can be used to predict carcinogenicity in humans. Many of these mouse liver carcinogens lack genotoxic activity and as such have been proposed to be tumor promoters. The limited amount of evidence for their tumor promoting activity is reviewed. Two mechanisms are reviewed that may explain the action of non-genotoxic carcinogens in mouse liver. These are 1) direct-action on precursor cancer cells, either to accelerate their growth or to prevent their death and 2) the selective growth advantage, resulting from regenerative hyperplasia of precursor cancer cells in response to the necrosis of normal cells produced by hepatotoxins. The estimation of human health risks on the basis of mouse liver tumor data is different for non-genotoxic carcinogens than for genotoxic carcinogens in 2 fundamental ways. The first involves intraspecies extrapolation and the second involves low-dose extrapolation. In conclusion, while mouse liver tumor data are seen to be of value in the estimation of human health hazard, it is of the utmost importance to distinguish between genotoxic and non-genotoxic mechanisms in applying such data.

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MOUSE LIVER TUMOR DATA: ASSESSMENT OF CARCINOGENIC ACTIVITY

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Significant numbers of chemicals have been shown to be carcinogenic in mouse liver although they do not exhibit carcinogenic activity in other organs or tissues of mice or rats. This review focuses on the reasons for the unique susceptibility of the mouse liver to these carcinogens and the extent to which the carcinogenic activity of a chemical in mouse liver can be used to predict carcinogenicity in humans.

Many of these mouse liver carcinogens lack genotoxic activity and, as such, have been proposed to be tumor promoters. Two mechanisms that may explain the action of nongenotoxic carcinogens in mouse liver are reviewed. These are: (1) direct action on precursor cancer cells, either to accelerate their growth or to prevent their death and (2) the selective growth advantage, resulting from regenerative hyperplasia of precursor cancer cells in response to the necrosis of normal cells produced by hepatotoxins.

Estimating human health risks on the basis of mouse liver tumor data is believed to differ for nongenotoxic and genotoxic carcinogens in two fundamental ways. The first involves intraspecies extrapolation and the second involves low-dose extrapolation. In conclusion, although mouse liver tumor data are seen to be of value in estimating human health hazard, it is important to distinguish between genotoxic and nongenotoxic mechanisms in applying such data. Further study of the biochemical and molecular mechanisms of chemical carcinogens is necessary to determine the relationship between their activity in mouse liver and their activity in humans.

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2. Key words: dose-response, genotoxic, mouse liver tumors, nongenotoxic, tumor promoters.

3. Abbreviations: 2-AAF, 2-acetylaminofluorene; CCl_4, carbon tetrachloride; DEHP, di-(2-ethylhexyl)phthalate; DNA, deoxyribonucleic acid; GGT, gamma-glutamyltranspeptidase; IARC, International Agency for Research on Cancer; MFO, mixed function oxidase; NCI, National Cancer Institute; NTP, National Toxicology Program; RNA, ribonucleic acid; TPA, 12-0-tetra-decanoylphorbol-13-acetate.

INTRODUCTION

Long-term bioassays, usually lifetime exposure to a chemical in rodents (mice and rats), are used to determine whether a substance is an animal carcinogen. Results from animal bioassays have then been used to identify chemicals that are suspected of being human carcinogens and to estimate human health hazards from these chemicals.

A list of chemicals that are carcinogenic in mouse liver is presented in Table 1. Significant numbers of these chemicals have been shown to be carcinogenic in mouse liver although they do not exhibit carcinogenic activity in other organs or tissues of mice or rats. Thus, if the mouse had been the only test species used, these carcinogens would not have been detected in organs other than the liver. Many of these chemicals are of interest because of their commercial and industrial applications and because of their presence in the environment, i.e., ambient air and drinking water. This review will focus on the reasons for the unique susceptibility of the mouse liver to these carcinogens and the extent to which the carcinogenic activity of a chemical in mouse liver can be used to predict carcinogenicity in humans.

GENOTOXIC VERSUS NONGENOTOXIC MECHANISMS

Chemical carcinogenesis is a prolonged and progressive process that can extend itself over a large portion of the lifetime of an animal. In some animal experiments, the sequence of chemical carcinogenesis from its beginning until the occurrence of cancer has been divided into two distinct stages: initiation and promotion. This operational division of chemical carcinogenesis was originally described by Berenblum (1941a,b) and Mottram (1944) in mouse skin, and has been subsequently described in most other organs and tissues (Lucier and Hook, 1983). The first demonstration of two-stage carcinogenesis in liver was reported by Peraino et al. (1971). In this study, phenobarbital was shown to promote tumors in 2-AAF-initiated rats. In addition, phenobarbital has subsequently been shown to be a promoter in mouse liver (Uchida and Hirono, 1979; Ward et al., 1983; Pereira et al., 1985).

Carcinogens that initiate carcinogenesis are believed to be genotoxic. It is assumed that chemical carcinogenesis is initiated by the interaction of a carcinogen or its metabolite with DNA, followed by fixation of the alteration or damage into the daughter genome during transcription. The adult rodent liver is characterized by a very low level of cellular proliferation, resulting in a very limited capacity for fixation of genotoxic alterations in DNA. For this reason, initiation of carcinogenesis in rodent liver usually requires a necrogenic dose of the carcinogen, which results in a regenerative cellular proliferation that transcribes the genotoxic alteration and completes the process of initiation.

The fixation of the alteration in the DNA must occur before the damaged DNA is repaired. The requirement that both DNA damage and cellular proliferation must

TABLE 1
Chemicals Carcinogenic in Mouse Liver and Their Carcinogenic Activity in Other Organs and Tissues of the Mouse and in the Rat

Chemical	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Aldrin	-	-	NCI, 1978a
Aminoanthraquinone, 2-	-	+	IARC, 1982a; NCI, 1978x
Aminobiphenyl, 4-	+	+	Soderman, 1982
Aminoazotoluene, O-	+	+	Soderman, 1982
Amino-1,4-dimethyl-5H-pyrido (4,3-b)indole, 3-	-	+	IARC, 1983b
Amino-9-ethylcarbazole	-	+	NCI, 1978p
Amino-2-methylanthraquinone, 1-	-	+	NCI, 1978t
Amino-1-methyl-5H-pyrido (4,3-b) indole, 3-	-	+	IARC, 1983b
Amitrol (amino-1H-1,2,4-triazole, 3-)	+	+	Soderman, 1982
Aramite	-	+	Soderman, 1982
Auramine	-	+	Soderman, 1982
Aurothioglucose	-	?	Soderman, 1982
Aziridine (ethyleneimine)	+	+	Soderman, 1982
Benz[a]anthracene	+	?	Soderman, 1982
Benzo[a]pyrene	+	+	Soderman, 1982
Benzidine	-	+	Soderman, 1982
Bis(2-chloroethyl)ether	+	-	Soderman, 1982
Bis-dimethylaminodiphenyl methane	-	+	Soderman, 1982
Bis(2-hydroxyethyl)dithiocarbamate, potassium	-	?	Soderman, 1982
Carbazole	+	?	IARC, 1984
Carbon tetrachloride	-	+	NCI, 1976a
Chloramben	-	-	NCI, 1977e

TABLE 1 - Continued

Chemical	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Chlordane	-	-	NCI, 1977b
Chlordecone (kepone)	-	+	Soderman, 1982
Chlorobenzilate	-	?	NCI, 1978k
Chloroform	-	+	NCI, 1976a
Chloro-m-phenylenediamine, 4-	-	+	NCI, 1978n
Chloro-o-phenylenediamine, 4-	-	+	IARC, 1982a, NCI, 1978l
Chloro-o-toluidine, 5-	+	-	NCI, 1979q
Chrysene	+	?	IARC, 1984
Chrysoidine	+	?	Soderman, 1982
Cinnamyl anthranilate	+	?	IARC, 1983b; NCI, 1980b
Cresidine-para	+	+	IARC, 1982a; NCI, 1979f
Cupferron	+	+	NCI, 1978
Cycasin	+	+	Soderman, 1982
Cyclochlorotine	+	?	Soderman, 1982
Cyclophosphamide	+	+	IARC, 1981
DDE, P,P'-	-	-	NCI, 1978v
Diallate	+	?	IARC, 1983a
Dibenzo(C,G)carbazole, 7H-	+	+	Soderman, 1982
Dichlorodibenzo-p-dioxin, 2,7-	+	-	NCI, 1979c
Dichloroethane, 1,2-	+	+	NCI, 1978h
Dichloro-p-phenylenediamine, 2,6	-	-	NCI, 1980e
Dicofol	-	-	IARC,1983a; NCI, 1978o
Dieldrin	-	-	NCI, 1978a,b
Di-(2-ethylhexyl)adipate	-	-	IARC, 1982b; NCI, 1980d

TABLE 1 - Continued

Chemical	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Di-(2-ethylhexyl)phthalate	-	+	IARC, 1982b; NCI, 1980g
Dihydrosafrole	+	+	Soderman, 1982
Dimethyl-4-aminoazobenzene, N,N-	+	+	Soderman, 1982
Dimethylhydrazine, 1,1-	+	-	Soderman, 1982
Dioxane, 1-4-	-	+	NCI, 1978m
Direct Black 38	+	+	IARC, 1982b
Dithiobiurea, 2,5-	-	-	Soderman, 1982
Ethyl-DDD, P,P'-	-	-	NCI, 1979j
Fluometuron	-	-	IARC, 1983a; NCI, 1980a
Griseofulvin	-	+	Soderman, 1982
Heptachlor	-	+	NCI, 1977c
Hepatachlor epoxide	-	?	Soderman, 1982
Hexachlorobenzene	+	-	Soderman, 1982
Hexachlorocyclohexane (lindane)	-	+	Soderman, 1982
Hexachlorodibenzo-p-dioxins	-	-	NCI, 1978j
Hexachloroethane	+	+	Soderman, 1982
Hydrazine (sulfate)	-	+	Soderman, 1982
Hydrazobenze	-	+	Soderman, 1982
Isosafrole	-	?	Soderman, 1982
Luteoskyrin	+	+	Soderman, 1982
Methylene bis(2 chloroaniline), 4,4'-	-	+	Soderman, 1982
Methylazoxymethanol	-	+	IARC, 1982a; NCI 1979p
4,4'-Methylene bis(N,N-dimethyl)benzenamine	+	+	NCI, 1979n
Michler's ketone	+	+	Soderman, 1982
Mirex	+	+	Soderman, 1982
Monuron	-	+	IARC, 1980
Nafenopin			

TABLE 1 - Continued

Chemicals	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Naphthylamine, beta-	-	-	Soderman, 1982
Naphthalenediamine, 1,5-	+	+	NCI, 1978w
1,5-Naphthalenediamine	+	?	IARC, 1982a
Nithiazide	+	+	IARC, 1983b; NCI, 1979g
Nitroacenaphthene, 5-	+	+	Soderman, 1982
Nitrobenzimidazole, 6-	-	-	NCI, 1979b
Nitroethylurea, N-	+	+	Soderman, 1982
Nitrofen	-	+	IARC, 1983a; NCI, 1978c; 1979o
Nitro-o-anisidine, 5-	-	+	NCI, 1978u
Nitro-p-acetophenetide, 3-	-	-	NCI, 1979e
Nitro-p-phenylenediamine, 2-	-	-	NCI, 1979m
Nitro-o-toluidine, 5-	+	-	NCI, 1978s
Nitrosodiethylamine, N-	+	+	Soderman, 1982
Nitrosodimethylamine, N-	+	+	Soderman, 1982
Nitrosodi-N-butylamine, N-	+	+	NCI, 1979r
Nitrosodiphenylamine, p-	-	+	Soderman, 1982
Nitrosomorpholine, N-	+	+	Soderman, 1982
Nitrosopiperidine, N-	+	+	Soderman, 1982
Nitrososarcosine, N-	+	+	Soderman, 1982
Norethisterone	+	+	Soderman, 1982
Norethisterone acetate	-	?	Soderman, 1982
Ochratoxin A	+	?	IARC, 1983b
Oxazepam	-	?	Soderman, 1982
Oxydianiline, 4,4'-	+	+	NCI, 1980c
Phenazopyridine HCl	-	+	NCI, 1978q

TABLE 1 - Continued

Chemical	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Phenicarbazide	+	?	Soderman, 1982
Phenobarbital, sodium	-	+	Soderman, 1982
Phenyl-2-naphthylamine, N-	-	?	Soderman, 1982
Piperonyl sulfoxide	-	-	NCI, 1979d
Polychlorinated biphenyls	-	+	Soderman, 1982
Ponceau MX	+	+	Soderman, 1982
Proflavine (3,6-acridinediamine)	-	+	NCI, 1977a
Propiolactone, beta-	+	+	Soderman, 1982
Rifampicin	-	-	IARC, 1980
Safrole	+	+	Soderman, 1982
Sudan I	+	-	Soderman, 1982
Tetrachlorodibenzo-p-dioxin, 2,3,7,8	+	+	NCI, 1980f
Tetrachloroethane, 1,1,2,2-	-	-	NCI, 1978d
Tetrachloroethylene	-	-	NCI, 1977d
Tetrachlorvinphos	-	+	IARC, 1983a; NCI, 1978e
Thioacetamide	+	+	Soderman, 1982
Thiodianiline, 4,4'-	-	+	IARC, 1982a; NCI, 1978g
Thiouracil	+	+	Soderman, 1982
Toluenediamine, 2,4-	+	+	NCI, 1979l
Toluidine, o-	-	+	NCI, 1979h
Toxaphene (stobane)	-	-	NCI, 1979a
Trichloroethylene	-	+	NCI, 1976b
Trichlorophenol, 2,4,6-	+	-	NCI, 1979l
Trifluraline	-	+	NCI, 1978f
Trimethylaniline, 2,4,5-			IARC, 1982a; NCI, 1979k

TABLE 1 - Continued

Chemical	Carcinogenic activity: Other organs of mice	Carcinogenic activity: Rats	Reference
Tris(2,3 dibromopropyl)phosphate	+	+	NCI, 19781
Uracil mustard	+	+	Soderman, 1982
Urethane	+	+	Soderman, 1982
Vinyl chloride	+	+	Soderman, 1982
Vinylidene chloride	+	+	Soderman, 1982
Zearalenone	+	-	IARC,1983b

occur for hepatocarcinogenesis to be initiated is evidenced by the inability of certain chemicals to initiate hepatocarcinogenesis. These chemicals include polycyclic aromatic hydrocarbons (benzo[a]pyrene and 7,12-demethylbenz[a]anthracene) (Craddock, 1976; Lutz et al., 1978; Eastman et al., 1978; Pereira et al., 1982; Tsuda et al., 1983) and methylating agents (N-methyl-N-nitrosoguanidine and N-methyl-n-nitrosourea) (Craddock, 1975 and 1976; Lawley, 1979; Pereira et al., 1983; Tsuda et al., 1980), which extensively bind hepatic DNA. These chemicals are not hepatocarcinogens unless they are administered to rats that are partially hepatectomized or intoxicated by CCl_4. It has been demonstrated that optimal initiation occurs when these types of chemicals are administered so that their maximum binding to DNA occurs during the regenerative hyperplasia induced by the partial hepatectomy (Ishikawa et al., 1980; Tsuda et al., 1980; Columbano et al., 1981; Pereira et al., 1983).

The sequential progression of the initiated cells to a focus of phenotypically altered cells, and then either to a carcinoma or to an adenoma that progresses to a carcinoma, can be greatly shortened using nongenotoxic chemicals. Nongenotoxic carcinogens, in contrast to genotoxic carcinogens, do not interact directly or indirectly after metabolism with cellular DNA to produce mutations and/or other alterations in the genome. Instead, they alter the phenotype of cells by interacting with cellular membranes, proteins or RNA. They might indirectly alter the structure of cellular DNA by affecting: (1) the methylation of cytosine in DNA or (2) the chromatin histones and proteins.

These nongenotoxic chemicals act as tumor promoters in two-stage experimental hepatocarcinogenesis. Such tumor promoters can also enhance the occurrence of tumors in animals that were not previously treated with an initiator by promoting the development of tumors from "spontaneously" initiated cells. Although nongenotoxic chemicals can be carcinogenic, they can only exert this activity in organs and tissues that possess either "spontaneously" or chemically initiated cells. Genotoxic carcinogens, on the other hand, can be "complete" carcinogens that can initiate and promote tumor appearance in organs and tissues that lack "spontaneously" initiated cells.

The spontaneous incidence of mouse hepatoma varies with the strain (Grasso and Hardy, 1975). The B6C3F1 mouse used in NTP studies has a tumor incidence in untreated controls of 31.1% in males and 6.2% in females (Tarone et al., 1981). The mouse liver carcinogens listed in Table 2 are either nongenotoxic or have very weak genotoxic activity and may act by promoting the "spontaneously" initiated cells responsible for the tumors in untreated B6C3F1 mice. The liver of untreated Fischer-344 and Osborn-Mendel rats (rat strains used in the NCI and NTP bioassay programs) have a much lower incidence of liver tumors than that of the B6C3F1 mouse (Sher et al., 1982) so that nongenotoxic mouse liver carcinogens would not be expected to be active as carcinogens in the liver of these rats. The nongenotoxic mouse carcinogens that lack carcinogenic activity in rat liver are presented in Table 2.

EVIDENCE FOR TUMOR PROMOTION

Although the chemicals listed in Table 2 have been proposed as tumor promoters in mouse liver, very few have actually been shown to promote mouse hepatocarcinogenesis. Phenobarbital has been shown to promote liver tumors initiated by either (1) dimethylnitrosamine in newborn mice (Uchida and Hirono, 1979), (2) ethylnitrosourea in weanling Swiss mice (Pereira et al., 1985), (3) diethylnitrosamine in weanling B6C3F1 mice (Ward et al., 1983) and (4) diethylnitrosamine in drinking water for 4 wk in B6C3F1 mice (Pereira et al., 1985). Diwan et al. (1984) obtained opposing results when administering phenobarbital at weaning to B6C3F1 mice that were initiated on day 15 of age with diethylnitrosamine. This procedure resulted in an inhibition of hepatocarcinogenesis. Except for this very interesting study, phenobarbital has been reported to promote the occurrence of both chemically and "spontaneously" initiated mouse liver tumors. DEHP is another mouse liver carcinogen that has been shown to promote liver tumors initiated by diethylnitrosamine in weanling B6C3F1 mice (Ward et al., 1983). DEHP and phenobarbital induced tumors in mice that were not previously treated with the initiator. These tumors could have resulted from promotion of "spontaneously" initiated hepatocytes. In a study sponsored by NCI, chloroform was shown to induce liver tumors in B6C3F1 mice (NCI, 1976a). It has been proposed that these tumors resulted from a nongenotoxic mechanism such as tumor promotion (Pereira et al., 1982; Reitz et al., 1982). We attempted to demonstrate the tumor-promoting activity of chloroform in mice initiated with ethylnitrosourea on day 15 after birth (Pereira et al., 1985). Instead of promoting the appearance of liver tumors, chloroform inhibited the appearance of both ethylnitrosourea-initiated and "spontaneous" liver tumors. A major difference between this study and the NCI-sponsored study was that we administered the chloroform in drinking water, whereas in the NCI study, the chloroform was administered in corn oil. The possible role of the different vehicles in producing the opposing results in these two studies is discussed later in the section on the mechanism of nongenotoxic carcinogens in mouse liver.

Kunz and co-workers (Tennekes et al., 1982; Kunz et al., 1983) used another approach to distinguish nongenotoxic from genotoxic carcinogens. This approach involved investigating the relationship between dose and time to tumor appearance. Previously, Druckrey and co-workers (Druckrey, 1967; Druckrey et al., 1967) established that for genotoxic carcinogens a relationship exists between the daily dose (D) and the median time to tumor appearance (T) such that $(D)(T)^n = K$. The value K is a constant. The value n is equal to or greater than 1 and is the acceleration factor of the number of required rare (mutagenic) events in the induction of cancer. Thus, a linear relationship is seen when the logarithm of the negative daily dose is plotted against the logarithm of the median time to the appearance of tumors. This linear relationship has been demonstrated for the following genotoxic carcinogens: diethylnitrosamine (Druckrey, 1967), diethanolnitrosamine (Druckrey et al., 1967), 4-dimethylaminoazobenzene (Druckrey, 1967), and N-nitrosomorpholine (Tennekes et al., 1982).

For the nongenotoxic mouse liver carcinogen, dieldrin, the relationship of the logarithm of the negative daily dose to the logarithm of the median time to tumor appearance was determined to be nonlinear (Tennekes et al., 1982; Kunz et al., 1983). At both the high- and low-dose ranges of the double logarithmic plot, changes in the daily dose of dieldrin did not significantly alter the time required for the appearance of tumors. It was only with the median doses of dieldrin that a relationship between the daily dose and the time-to-tumor appearance was obtained. The nonlinear dose vs. time-to-tumor relationship for dieldrin suggests that its carcinogenicity is determined by the daily dose and exposure duration and not, as with genotoxic carcinogens, by the sum of consecutive doses. Furthermore, this nonlinear relationship for dieldrin implies an activity that is (1) reversible, (2) less at low doses than would be predicted from higher doses and (3) saturable at very high doses.

The relationship between the daily dose and the median time to the appearance of tumors needs to be determined for many more nongenotoxic carcinogens to demonstrate whether the relationship observed for dieldrin is representative of this class of carcinogens. Because of the insensitivity of tumor incidence to dose, especially at low doses, and because a large number of animals are needed for the requisite serial sacrifices, a more sensitive and earlier marker of carcinogenic activity would be of great value. Altered foci such as basophilic foci (Goldfarb et al., 1983) and iron-deficient foci (Williams and Watanabe, 1978), when validated as quantitative indicators of carcinogenic activity, could be used in these studies as indicators of tumorigenic response.

MECHANISMS OF NONGENOTOXIC CARCINOGENS

Several mechanisms may explain the action of nongenotoxic carcinogens in mouse liver. Two possible mechanisms are: (1) direct action on precursor cancer cells, either to accelerate their growth or to prevent their death and (2) the selective growth advantage, resulting from regenerative hyperplasia of precursor cancer cells in response to the necrosis of normal cells produced by hepatotoxins.

In the case of the first mechanism, nongenotoxic carcinogens would be mitogenic to liver at doses that do not cause necrosis to the extent required for a regenerative hyperplasia. Nongenotoxic chemicals that are believed to be tumor promoters in mouse liver because of their mitogenic activity are listed in Table 3. These chemicals at nonnecrogenic doses have been shown to stimulate DNA synthesis and/or mitogenesis.

Schulte-Hermann and co-workers (Schulte-Hermann, 1974; Schulte-Hermann et al., 1983) and Peraino et al. (1975) have shown that the stimulation of the rate of DNA synthesis and mitogenesis rat liver by tumor promoters lasted only for a few days, after which these rates returned to pre-exposure levels despite the continued exposure to the promoter. These results indicate that in rodent liver, there is a mechanism of feedback inhibition that prevents the continuous stimulation of DNA synthesis, mitosis and liver growth by tumor promoters. The liver mass increased by approxi-

TABLE 2

Mouse Liver Carcinogens that Appear to Possess Little or No Genotoxic Activity[a]

Aldrin*[b]
Aminoanthraquinone, 2-
Amitrol
Auramine
Bis(2-hydroxyethyl)dithiocarbamate*
Carbazole
Carbon tetrachloride
Chlorobenzilate*
Chlordane*
Chlodecone
Chloroform
Cinnamyl anthranilate
Cycasin
Cyclochlorotine
DDE, P,P'-*
Dichlorodibenzo-p-dioxin, 2,7-
Dicofol*
Dieldrin*
Di(2-ethylhexyl)adipate*
Di(2-ethylhexyl)phthalate
Dihydrosafrole
Dioxane 1,4-
Ethyl-D,D,D, P,P'-*
Fluometuron*
Griseofulvin*
Heptachlor
Heptachlor epoxide
Hexachlorobenzene*
Hexachlorocyclohexane
Hexachlorodibenzo-p-dioxins
Hexachloroethane*
Isosafrole
Luteoskyrin*
Mirex
Monuron
Nitrobenzimidazdole, 6-*
Nitrofen (TG)*
Norethisterone
Norethisterone acetate
Ochratoxin A
Phenazopyridine HCL
Phenicarbazide
Phenobarbital
Phenyl-2-napthylamine, N-*
Piperonyl sulfoxide*
Polychlorinated biphenyls
Ponceau MX
Rifampicin*
Safrole
Sudan I
Tetrachlorodibenzo-p-dioxin 2,3,7,8-
Tetrachloroethylene*
Tetrachlorvinphos
Thioacetamide
Trichloroethylene*
Trichlorophenol 1,2,4,6-
Trifluraline (TG)
Zearalenone

[a]Chemicals that induced liver tumors in mice and either lacked genotoxicity or possessed very weak genotoxicity, so that they are believed to act by a nongenotoxic mechanism. References pertaining to the lack of genotoxicity of these chemicals can be found in Monographs Vol. 1-32 and supplement 4 and in Soderman, 1982.

[b]The chemicals marked by asterisks were not carcinogenic in all organs of the mouse other than the liver, nor were they carcinogenic in the rat.

mately 40% and remained at that size until the exposure to the tumor promoter ceased, after which the liver mass returned to normal within a few days (Schulte-Hermann et al., 1983). Hence, the continuous exposure to the promoter is required to prevent the loss (death) of normal hepatocytes that had previously been stimulated to proliferate.

The preferential stimulation of the growth of precursor cancer cells by tumor promoters could result from the resistance of these cells to the feedback inhibition of cell proliferation that was exhibited by normal hepatocytes. Schulte-Hermann et al. (1983) have shown in rat liver that GGT foci initiated by a single dose of N-nitrosomorpholine have a higher rate of DNA synthesis and mitosis than that of

surrounding cells. Intermittent treatment (once a week) with the proposed tumor promoters cyproterone acetate or progesterone caused a preferential enhancement of DNA synthesis in the foci at each time investigated up to 6 mo treatment. However, in another experiment in which GGT foci were also initiated by N-nitrosomorpholine, continuous exposure to either phenobarbital or α-hexachlorocyclohexane in the diet resulted in only an initial enhancement of DNA synthesis in the foci cells. Therefore, it would appear that the hepatocytes in GGT foci are still susceptible to feedback inhibition of DNA synthesis, even though the level of DNA synthesis in foci cells is higher than in surrounding cells. The critical importance of these studies warrants their repetition in both mice and rats using other tumor promoters and using markers other than GGT for altered foci to prove that precursor cancer cells are still sensitive to feedback inhibition of cell proliferation.

Whether the existence of preneoplastic/neoplastic lesions depends on the continued exposure to the promoter has not been investigated in mouse liver, but it has been investigated in rats. Schulte-Hermann et al. (1983) have demonstrated in N-nitrosomorpholine-initiated and phenobarbital- or α-hexachlorocyclohexane-promoted rats, that when the administration of the promoter was ceased, the number of GGT foci and the average size of the foci decreased and approached the values for N-nitrosomorpholine-initiated, but not promoted, animals. This decrease in the number of foci cells could be the result of either cell death or a remodeling of GGT-positive cells to GGT-negative cells. Herren and Pereira (1983) have shown in rat liver that diethylnitrosamine-initiated GGT foci promoted with phenobarbital did not regress when the phenobarbital treatment was stopped. The reason for these opposing observations on the stability of GGT foci needs to be determined.

The existence of promoter-dependent and promoter-independent precancerous lesions has been studied in only a few other models. Herren-Freund et al. (1985), using the Solt-Farber procedure in rats treated with 2-AAF in the diet for 2 wk (Solt and Farber, 1976; Solt et al., 1977), reported that the remodeling and regression of diethylnitrosamine-initiated GGT foci and nodules were prevented by subsequent administration of phenobarbital. Burns and Albert (1982) reported that mouse skin promotion by TPA results in both promoter-dependent and promoter-independent papillomas. These results indicate the existence in rat liver and mouse skin of promoter-dependent precursor cancer cells. It is thus reasonable to speculate that tumor promoters in mouse liver might induce promoter-dependent and promoter-independent lesions and might prevent the regression (cell death) and remodeling of these precancerous lesions.

In summary, the mechanism by which nongenotoxic carcinogens directly enhance the growth of precursor cancer cells in mouse liver is not known. However, sufficient evidence, mainly from rat liver studies, exists to warrant further study of the following mechanisms: (1) enhancement of DNA synthesis and mitosis, with the loss by the precursor cancer cells of the feedback inhibition to cell proliferation, (2) prevention of the death of precursor cancer cells, which have a greater rate of proliferation than

nonprecursor cells and (3) prevention of remodeling and regression of promoter-dependent precursor cancer cells.

The second general mechanism that may explain the activity of nongenotoxic carcinogens in mouse liver is the selective growth advantage of precursor cancer cells over normal cells when exposed to hepatotoxins. This mechanism of promotion has been investigated mainly in rat liver and not in mouse liver. In rat liver, precancerous cells have been shown to be resistant to the toxicity of hepatotoxins (Laishes et al., 1980; Carr and Laishes, 1981). This finding is the basis for the Solt and Farber model for chemical carcinogenesis (Solt and Farber, 1976; Solt et al., 1977), in which rats that have been previously treated with an initiator such as diethylnitrosamine, received 2-AAF in their diet for 2 wk. One week after the animals began receiving the 2-AAF in their diet, they also received either a two-thirds partial hepatectomy or a necrogenic dose of CCl_4 as a regenerative stimulus (Tsuda et al., 1980; Solt et al., 1983). One week after the cessation of the 2-AAF diet, the liver of animals that had received an initiator before beginning the 2-AAF regimen contained numerous GGT foci and nodules. These lesions are believed to result from precancerous cells that were resistant to the toxicity of 2-AAF and thus were stimulated to grow by the regenerative stimulus. The greater toxicity of 2-AAF to noninvolved cells prevented them from responding to the same regenerative stimulus.

Precancerous cells in rat liver have been shown both *in vivo* and *in vitro* to be resistant to the toxicity of chemicals. Nodules in rat liver have a reduced ability to metabolize chemical carcinogens (Gravela et al., 1975; Cameron et al., 1976; Astrom et al., 1983; Farber, 1984) resulting in a decreased binding of the carcinogen to DNA (Farber et al., 1976). Precancerous liver cells in GGT foci and nodules have an increased level of glutathione, which might be related to their resistance to the toxicity of chemicals (Demi and Oesterie, 1980). Hepatocytes isolated from rat liver containing nodules, compared to hepatocytes from control animals, have a reduced susceptibility in primary culture to the cytotoxicity of many hepatocarcinogens (Laishes et al., 1980; Carr and Laishes, 1981). This reduced susceptibility of precancerous cells to the toxicity of chemicals would tend to give them a selective advantage in responding to the regenerative stimuli of hepatotoxins.

Mouse liver carcinogens such as CCl_4, chloroform and trichloroethylene are hepatotoxins that might act by inducing a selective regenerative hyperplasia in precancerous cells. In NCI-sponsored studies, CCl_4 (NCI, 1976a), chloroform (NCI, 1976a), 1,1,2-trichloroethane (NCI, 1978y), and trichloroethylene (NCI, 1976b), when administered in corn oil to mice by stomach gavage, induced liver tumors. In another study, chloroform administered in the drinking water inhibited the occurrence of both chemically initiated and "spontaneously" initiated liver tumors (Pereira et al., 1985). Corn oil has been shown to increase liver MFO activity (Norred and Wade, 1972; Newberne et al., 1979). Other inducers of MFO activity have been found to increase the hepatotoxicity of chloroform (Pohl, 1979). Chloroform hepatotoxicity has been shown to result in a regenerative hyperplasia (Reitz et al., 1982). An increase in the

hepatotoxicity of chloroform when administered in corn oil, compared to when administered drinking water, could result in a greater level of regenerative hyperplasia and thus in an increase in the hepatocarcinogenicity of chloroform. The inhibition of ethylnitrosourea-initiated liver tumors by chloroform administered in drinking water could result from a low-level hepatotoxicity that was not sufficient to cause a regenerative hyperplasia. There are many other possible explanations for this opposing effect of chloroform, but these explanations are beyond the scope of this review. However, the apparent effect of vehicle (corn oil vs. drinking water) and/or route of administration on the carcinogenic activity of halogenated hydrocarbons in mouse liver, has critical implications on the use of mouse liver tumor data for predicting carcinogenic activity in humans.

In summary, some carcinogens that are nongenotoxic in mouse liver possess hepatotoxicity that can cause a regenerative hyperplasia. This hyperplasia might result in the selective proliferation of precancerous cells that are resistant to the toxicity of these chemicals.

IMPLICATIONS FOR EXTRAPOLATION BETWEEN SPECIES AND AT LOW DOSES

The procedure for estimating the human health risks to chemical carcinogens using results from experiments in laboratory animals is believed to differ for nongenotoxic and genotoxic carcinogens in two fundamental ways. The first involves intraspecies extrapolation and the second involves low-dose extrapolation.

Intraspecies extrapolation of nongenotoxic mouse liver carcinogens is complicated by the apparent requirement for either spontaneously or environmentally initiated precancerous cells. The level of both spontaneously and environmentally initiated cells is expected to vary with species, resulting in species specificity for nongenotoxic carcinogens. Mice, as indicated by their higher spontaneous level of liver tumors (Tarone et al., 1981; Sher et al., 1982), would appear to have more spontaneously initiated liver cells than rats. Besides species specificity, nongenotoxic carcinogens also exhibit target organ specificity, probably in part because of the requirement for initiated cells to be present and in part because of organ specificity for the receptor of the nongenotoxic carcinogen. Nongenotoxic mouse liver carcinogens would therefore be expected to be inactive in human organs or tissues other than the liver unless the appropriate initiated cells and receptor are present. Evidence suggests that nongenotoxic and androgenic anabolic steroids (IARC, 1979) and estrogens used in oral contraceptive drugs (Baum et al., 1973; Edmondson, 1976; Barrows and Christopherson, 1983) induced adenomas in humans, which implies the presence of initiated cells. The above results in humans indicate that nongenotoxic carcinogens are active in humans. Therefore, the mouse liver tumor data on nongenotoxic carcinogens appear to be appropriate for indicating potential human cancer (tumor) risk. As a consequence of the requirement by nongenotoxic carcinogens that initiated cells be present, the

relative level of initiated cells in human and mouse liver needs to be estimated as part of the extrapolation of mouse liver tumor data to human cancer risk.

When considering low-dose extrapolation, the difference between nongenotoxic and genotoxic carcinogens relates to the probable break and/or threshold in the dose-response curve for nongenotoxic carcinogens, as compared to the linear dose-response curve for genotoxic carcinogens. A threshold for nongenotoxic carcinogens that acts by virtue of regenerative hyperplasia is expected because the dose of the carcinogen must be sufficient to produce hepatotoxicity severe enough to produce a regenerative hyperplasia.

The nongenotoxic carcinogens that act by being mitogenic and/or by causing a decrease in cell death and remodeling, are also expected to have a sigmoidal curve. The sigmoidal dose-response curve would result from (1) the characteristics of the receptor, enzyme or membrane sites with which the carcinogen must interact and (2) the requirement of the carcinogen to interact with a finite number of sites before an effect can be elicited. The only example of a dose-response study for nongenotoxic carcinogens is the work of Kunz and co-workers (Tennekes et al., 1982; Kunz et al., 1983), who demonstrated a break in the double logarithmic curve of the daily dose of dieldrin and the median time to tumor. This result is at variance with the linear curves obtained for genotoxic carcinogens (Druckrey, 1967; Druckrey et al., 1967; Tennekes et al., 1982). The linear nature of the curves for genotoxic carcinogens could result from the stochastic property of their interaction with DNA, an interaction that is dependent on the probability of binding to a critical site in the DNA and not, as with nongenotoxic carcinogens, on the requirement for a certain number of interactions. Therefore, although the dose-response curve for genotoxic carcinogens might be linear, the dose-response curve for nongenotoxic carcinogens is expected to be nonlinear. This difference in dose-response curves does not mean that nongenotoxic carcinogens do not represent a risk to human health, but rather that a safe level of exposure to nongenotoxic carcinogens might exist.

IMPORTANCE OF MOUSE LIVER TUMOR DATA IN DETECTING POTENTIAL HUMAN CARCINOGENS: CONCLUSIONS

Mouse liver tumor data are important for detecting nongenotoxic carcinogens that might be active in humans and that might not be detected in rat liver. The insensitivity of rat liver could result from a low level of "spontaneously" initiated cells. The nongenotoxic carcinogens in mouse liver might be active in rat liver following initiation by a genotoxic carcinogen. Initiation-promotion studies should be performed in rat liver to determine whether nongenotoxic carcinogens in mouse liver are tumor promoters in rat liver, and thus are also likely to be promoters in human liver. Since human liver might contain "spontaneously" or environmentally initiated cells, nongenotoxic mouse liver carcinogens might be active (carcinogenic) in humans.

Mouse liver tumor data are also important because they can aid in detecting genotoxic carcinogens that are missed in rats. Examples of genotoxic carcinogens detected in mouse liver, but not in rats, include bis(2-chloroethyl)ether, chrysoidine, 1,1-dimethylhydrazine, beta-naphthylamine, 2-nitro-p-phenylenediamine and 1,1,2,2-tetrachloroethane. Mouse liver tumor data are additionally useful in estimating the range of carcinogenic potency a genotoxic chemical might have in different species. For example, aflatoxin B_1 is a very potent carcinogen in rat liver, whereas mouse liver is relatively resistant to this substance (Wogan, 1976). If the reason for these differences in sensitivity between the mouse and the rat can be understood, it may be possible to determine whether the sensitivity of humans resembles that of the mouse or that of the rat, or whether neither species is appropriate for use in estimating the risk to humans.

In conclusion, although mouse liver tumor data are seen to be of value in estimating human health hazard, it is important to distinguish between genotoxic and nongenotoxic mechanisms in applying such data. Further study of the biochemical and molecular mechanisms of chemical carcinogens is necessary to determine the relationship between their activity in mouse liver and their activity in humans.

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